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Effectiveness Test on *Centella asiatica* Extract on Increasing Elasticity Level on Male Mus Musculus

Celvin Angkasa[a], Alexander Angkasa[b], I Nyoman Ehrich Lister[c], Ermi Girsang[d], Edy Fachrial[e*]

[a,b]*Master of Biomedical Science, Faculty of Medicine, Prima Indonesia University, Medan, North Sumatera, Indonesia*

[c]*Department of Physiology, Faculty of Medicine, Prima Indonesia University, Medan, North Sumatera, Indonesia*

[d]*Faculty of Public Health, Prima Indonesia University, Medan, Indonesia*

[e]*Laboratory of Biomoleculer, Faculty of Medicine, Prima Indonesia University, Medan, Indonesia*

[a]*Email: Celvin_angkasa@yahoo.com*

Abstract

Gotu kola (Centella asiatica), is one of the plants with a potential development in dermatocosmetology, because it contains triterpenoid compounds, asiaticoside, madecassoside, flavonoids, efficacious as an antioxidant and prevent skin premature aging. The aim of this study was to compare the effectiveness of anti-aging extract of Centella asiatica to increase elasticity levels. Phytochemical test results of extracts contain alkaloids, tannins, triterpenoid, flavonoids, glycosides. The results showed that the research data were abnormally distributed ($p < 0.05$), the average highest measurement of skin elasticity levels was found in the 10% extraction treatment group. The difference in the average measurement of significant elasticity levels at weeks 2, 3, and 4 were $p < 0.005$ (p0,045; p0,008; p0,009). The difference in average elasticity level will increase significantly ($p < 0.005$) in line with the length of time spent. Cream with 10% of Centella asiatica extract showed an increase in skin elasticity levels by 40.58%. The multiple linear regression test, the greatest Pearson correlation value at week 4 on all measurements was found in comparison with week 3, elasticity levels (94.2% p0,000) and the results of the correlation value increased significantly with the addition of treatment time ($p < 0.005$). In conclusion, the results of this study indicate the administration of Centella asiatica extract increased the average of elasticity level compared to the control group, positively correlated with the increase of the duration and the quantity of Centella asiatica extract were given.

Keywords: Centella asiatic; Anti-aging; Elasticity.

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* Corresponding author.

1. Introduction

As the widest and outermost organ, skin can also protect body from the outside environment. This protection function occurs by biological mechanisms, such as a continuous formation of keratin substance layer, cell respiration, regulation of body temperature, production of sebum, sweat, as a sense of taste and touch, and defense against pressure and infection from outside [1]. However, this function can be disrupted because of an imbalance between oxidants and antioxidants which influence by several factors such as Reactive Oxygen Species (ROS), solar ultraviolet (UV), and temperature resulting in cell damage and skin aging [2, 3, 4]. Based on these phenomena, one of the solution that can be considered is utilizing natural sources. Gotu Kola *(Centella asiatica)* is one of the natural sources that have antioxidant activity [5]. Beside that, the compounds also can reduce oxidative stress, stimulate cell regeneration, hydration, and increase cell viability [6, 7]. Levels of elasticity, hydration, and also sebum are anti aging parameters that are quite commonly used. In addition, other anti aging parameters can also be used such as collagen levels, sensitivity, blemishes, wrinkles, and pore size. The aim of this study is to investigating the effectiveness of *Centella asiatica* extract on increasing elasticity levels.

2. Material and Methods

2.1. Materials

Aquades, Glacial Acetic Acid, Hydrochloric Acid 2N, Sulfuric Acid 2N, Sulfuric Acid, Ethanol 70%, FeCl3 10%, Isopropanol, Bouchardat reagent, Dragendorff reagent, Meyer reagent, Molisch reagent, Lead (II) acetate, gotu kola.

2.2. Instruments

Measuring cup, Glass beaker, Test tube, Pestle, Aluminum foil, Stirring rod, Blender, Porcelain cup, Funnel, Filter paper, Drying cupboard, Mortar, Analytic balance, Water Bath, Maceration bottle, Drip pipette, Rotary Evaporator, Spatula.

2.3. Extract Manufacture

The natural ingredients used are gotu kola herbs. Gotu kola washed and then dried in a drying cupboard for 3 days (72 hours) with a temperature of ± 40^{o}C to dry which is characterized by the simplicia easily broken [8]. The simplicia is weighed, blended until becomes a simplicia powder and then put into plastic bags, labeled and stored in a dry place [9]. At least 10 parts of gotu kola simplicia (1 kg of simplicia powder) soaked in 75 parts of 70% ethanol (7.5 liters) liquid in a maceration vessel and then closed, left for 5 days protected from light while stirring every day. The macerate is filtered, then the filtrate is macerated again with 25 parts of 70% ethanol (2.5 liters) quarantine liquid in a closed vessel, left in a cool place, protected by light for 2 days then filtered. The macerate obtained was concentrated by concentrating with a rotary evaporator at a temperature of 40^{o}-50^{o}C until a thick extract was obtained [10].

2.4. Phytochemical Methods

- ***Alkaloid Test***

Each simplicia and extract ± 0.5 g were added 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated over a water bath for 2 minutes, cooled and filtered. The filtrate obtained was used for the alkaloid test. 3 test tubes were taken, then 0.5 ml of filtrate was inserted into it. In each test tube 2 drops of Mayer reagent, Bouchardat reagent and Dragendorff reagent were added [11].

- ***Tannins Test***

Each simplicia and extract ± 0.5 g, run with 10 ml of water then filtered, the filtrate is diluted with water until it is colorless. 2 ml of solution is added 1-2 drops of 1% iron (III) chloride reagent [11].

- ***Saponin Test***

Each simplicia and extract ± 0.5 g were put into a test tube, then added 10 ml of hot water, then cooled, and shaken vigorously for 10 minutes [11].

- ***Flavonoid Test***

Each simplicia and extract ± 0.5 g were added with 20 ml of hot water, boiled for 10 minutes and filtered in hot conditions, into 5 ml filtrate were added 0.1 g of magnesium powder and 133 ml of hydrochloric acid concentrated and 2 ml of amyl alcohol, shaken and allowed to separated [11].

- ***Triterpenoid Test***

Each simplicia and extract ± 1 g, macerated with 20 ml of hexane for 2 hours, filtered. The filtrate was evaporated in the vaporizer cup and the remaining Liebermann-Burchard reagent was added through the cup wall [11].

- ***Glycosides Test***

Each extract ± 3 g was filtered with 30 ml of technical ethanol mixture with water (7: 3) refluxed for 10 minutes, cooled and filtered. Then 20 ml of filtrate was added with 25 ml of water and 25 ml of lead (II) acetate 0.4 M, shaken, allowed to stand for 5 minutes then filtered. The filtrate was carried out with 20 ml mixture of chloroform and isopropanol (3: 2), repeated 3 times. Then evaporated at 50^{o}C. The remainder is dissolved in 2 ml of methanol. The remainder is dissolved in 2 ml of methanol. The remaining solution used for the experiment was put in a test tube and evaporated over a water bath. The remaining 2 ml of water is added with 5 drops of molish reagent. Then slowly adding 2 ml of concentrated sulfuric acid through the tube wall [11].

2.5. Creams Formulation

Cream preparations are made based on a standard formula that has been modified using a basic type of oil cream in water [12].

Table 1: Creams Formulation

Ingredients	Concentration (%)				
	F0	**F1**	**F2**	**F3**	**F4**
Gotu Kola extract	-	2,5	5	7,5	10
Stearic acid	15	15	15	15	15
Setil alcohol	10	10	10	10	10
Vaselin	10	10	10	10	10
Mineral oil	12	12	12	12	12
Isopropyl palmitate	12	12	12	12	12
Glycerin	5	5	5	5	5
Triethanolamine	1	1	1	1	1
Fragrance	q.s	q.s	q.s	q.s	q.s
Preservative	q.s	q.s	q.s	q.s	q.s
Water	ad 100	ad 100	ad 100	ad 100	ad 100

2.6. Animal Trial Procedure

Anti-aging activity test was carried out on 25 male mice samples and divided into 5 groups. Group 1 treated by using F0 (0% extract), group 2 treated by using F1 (2.5% extract), group 3 treated by using F2 (5% extract), group 4 treated by using F3 (7.5% extract), group 5 treated by using F4 (10% extract). Mice are placed in cages with a size of 40 cm x 20 cm x 10 cm, temperatures 25-27^{o}C, based on wooden husks that are replaced every other day, light comes from the window during the day, and cross ventilation on the top of the cage. 1 cage filled with 5 mice. All mice were acclimatized for 7 days in order for the test animals to be able to adapt to the environment. All mice will be shaved on the back of an area of 2x2 cm2 using a manual shaver. Then the level of elasticity of the test animals before treatment was examined by Skin Analyzer EH 900 U. After measuring the initial skin condition, the treatment starts with applying the cream to the marked area. The treatment is applied twice a day for 4 weeks. Elasticity levels were measured every week for 4 weeks using Skin Analyzer EH 900 U.

2.7. Statistical analysis

All results were expressed as mean±standart deviation for each concentration of sample. Statistical analysis was performed by using Shapiro-Wilk test, Kruska-Wallis test, Mann-Whitney test, and Multiple Linear Regression test as a further analysis. Differences were accepted as statistically analysis at $P < 0.05$.

3. Result

3.1. Phytochemical test

Phytochemical test results showed that the extract contained alkaloids, flavonoids, triterpenoids, tannins, and glycosides.

3.2. Saphiro-Wilk test

The research data has been tested for normality of the data using the Saphiro-Wilk test and The results showed that the research data were abnormally distributed ($p < 0.05$). The average measurement of elasticity levels in all treatment groups can be seen in table 2.

Table 2: Average Measurement of Elasticity Levels in All Treatment Groups

Elasticity Measurement Result										
	Initial		**Week 1**		**Week 2**		**Week3**		**Week4**	
	N=25		**N=25**		**N=25**		**N=25**		**N=25**	
	M	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
F0	45,2	3,27	45,2	3,27	45,2	3,89	45,8	3,70	46,2	3,33
F1	47,8	1,92	48,4	1,94	50,2	1,78	52	1,41	53	1,58
F2	45,6	1,51	46,8	1,92	48,6	2,07	52	1,41	52	1,58
F3	47	2	48	2,23	51	2,23	50,6	0,89	60,4	2,40
F4	47,8	2,04	50	2,44	53	2,34	58	1,58	67,2	2,28

Based on table 2, the average measurement of the highest level of skin elasticity found in the 10% extraction treatment group**.**

3.3. Kruska-Wallis test

The average difference in measurement of elasticity levels between groups in each measurement of sample data can be seen in table 3 which was analyzed by Kruska-Wallis test.

Table 3: Differences Measurement of Elasticity Levels Between Treatment Groups on Each Measurement

	P* Value				
	Initial	**Week 1**	**Week 2**	**Week3**	**Week4**
Elasticity	0,171	0,112	0,045	0,008	0,009

Based on table 3, there were differences in the measurement of elasticity levels which found a significant difference at weeks 2, 3, and 4 $p < 0.005$ (p0.045; p0.008; p0.009)

3.4. Man-Whitney test

The average difference in the measurement of elasticity levels between groups F0 and other groups can be seen in Table 4

Table 4: Differences in Measurement of Average Elasticity levels Between Groups F0 and Other Treatment Groups.

P Value					
Groups	**Initial**	**Week 1**	**Week 2**	**Week3**	**Week 4**
F0-F1	0,171	0,112	0,045	0,008	0,009
F0-F2	0,670	0,344	0,206	0,018	0,015
F0-F3	0,292	0,171	0,027	0,008	0,009
F0-F4	0,171	0,044	0,016	0,009	0,008

From Table 4, can be concluded that the average difference in elasticity levels will increase significantly ($p < 0.005$) with the addition of treatment time. The difference in the increase in elasticity levels was found at weeks 3 and 4 compared to the initial treatment. The extraction level which showed a significant difference was seen in 10% extraction group.

3.5. Elasticity Percentage Levels

Table 5: The Average Elasticity Percentage Increase from Week 1 to Week 4 in All Treatment Groups

Groups	**Initial**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**% Elasticity Increased**
F0	45.2	45.2	45.2	45.8	46.2	2.2
F1	47.8	48.4	50.2	52	53	10.88
F2	45.6	46.8	48.6	50.6	52	14.03
F3	47	48	51	55.8	60.4	28.51
F4	47.8	50	53	58	67.2	40.58

Based on table 5 and figure 1, showed that cream with or without extract still increases the elasticity levels for four weeks of treatment. However, the best results were observed in F4 group (10% *Centella asiatica* extract) where the extract increase the elasticity level by 40.58% with an average final score by 67.2 from 47.8, followed by F1 group (2.5% *Centella asiatica* extract) which showed the lowest increase by 10.88% with an average final score by 53 from 47.8 compared to the other groups with extract. On the other hands, group F0 (0% *Centella asiatica* extract) showed the increase by 2.2% with and average final score by 46.2 from 45.2 after four weeks of treatment.

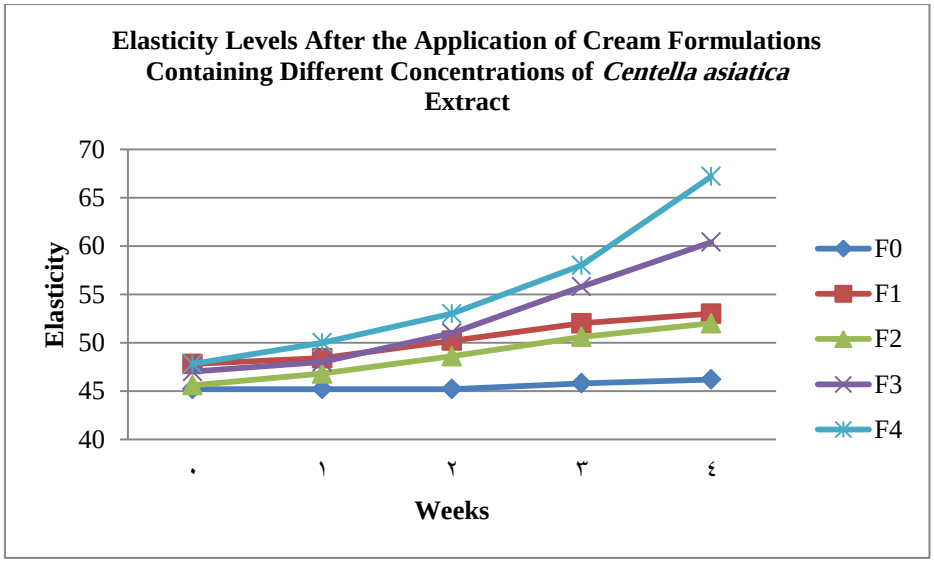

Figure 1: Graphics of Elasticity Levels on Every Weeks Measurement

3.6. Multiple Linear Regression test

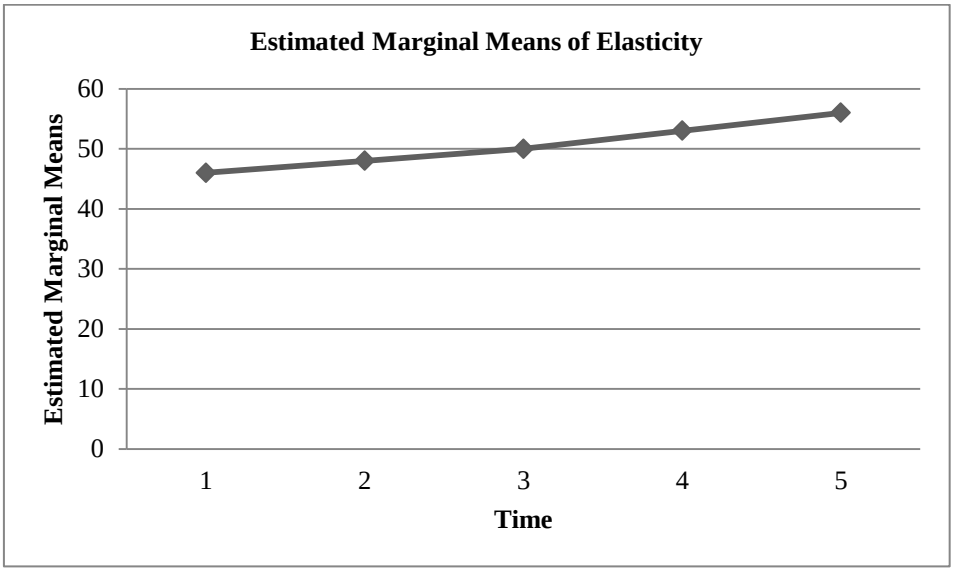

Figure 2: Plot of Multiple Linear Regression Tests on Average Elasticity Levels

The multiple linear regression test, the greatest Pearson correlation value at week 4 on elasticity levels measurement was found in comparison with week 3 (94.2% p0,000) and the correlation value increased significantly with the addition of treatment time ($p < 0.005$).

4. Discussion

Phytochemical test results showed that the extracts contain alkaloids, tannins, triterpenoid, flavonoids, glycosides. Scientific research has proven that there are various biochemical components found in gotu kola. The most active biological compounds (triterpenes) and the most important are asiatic acid, madecasic acid,

asiaticoside and madecassoside [13]. This study has a similar result was reported by Yulianti and his colleagues [14] which mentions that the asiaticoside in *Centella asiatica* increased the proliferation induction in dermis fibroblast cells much better than retinoic acid, where the fibroblast proliferation will increase levels of elastin and collagen types I and III, due to the content of asiaticoside that are able to regenerate and also increase levels of collagen which will ultimately help the formation of elastin which serves to increase elasticity so that skin shrinkage is reduced. The administration of *Centella asiatica* 50 mg extract orally increased the amount of collagen more and reduced MMP-1 expression than vitamin C 9 mg in male Galur Wistar exposed to UVB rays was observed by Herawati and his colleagues [15], The similarity of Herawati and his colleagues study with this study was that there was an increase in anti-aging parameters after the administration of Centella asiatica extract, and was observed for 4 weeks. The difference in Herawati and his colleagues study with this study was Herawati and his colleagues study used 30 subjects of male Galur Wistar, weight 180-200 grams, aged 10-12 weeks, the test group divided into 3 groups, positive controls using vitamin C, Gotu kola leaves are used as extract, extract was given orally 50 mg, while the oral dose of vitamin C was 9 mg, using the parameters of decreasing MMP-1 expression to measure anti-aging activity. whereas this study used 25 subjects of male Mus Musculus, weight 25-30 grams, aged 10-12 weeks, the test groups divide into 5 groups, Gotu kola herb are used as extracts, extract was given by topically applied and used elasticity level as anti-aging parameters. In addition to its ability to help synthesize proteins to form collagen, Centella Asiatica also functions to restore elasticity, elasticity of the skin, so it is widely used for beauty products for anti-aging [14]. Elastin provides elastic tissue with the ability to stretch and stretch and plays an important role in supporting and maintaining other healthy cells [16]. Histologically, elastin fibers are divided into three groups, namely oxytalan, elaunin, and elastic. Oxytalan is on the outer surface, very thin, and extends from the perpendicular to the dermal-epidermal junction. Elaunin and elastic are in a deeper and thicker layer. When the skin experiences photoaging, elastin changes its shape and function into elastosis tissue, where elastin fibers turn thick and irregular. Elastosis tissue can lead to clinical manifestations of aging of the skin, namely the skin looks loose or has reduced elasticity [4].

5. Conclusion

This study indicate the administration of *Centella asiatic*a extract increased the average of elasticity level compared to the control group, positively correlated with the increase of the duration and the quantity of *Centella asiatica* extract were given.

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